REMARKS

This application is a continuation of U.S. Serial No. 09/353,407, filed July 15, 1999. This continuation application is filed to obtain coverage for embodiments disclosed in the specification as originally filed.

This continuation application claims the benefit of the prior filed, copending parent application, United States Serial No. 09/353,407, filed July 15, 1999. Accordingly, pursuant to 37 C.F.R. § 1.78(2), Applicants have amended the specification to contain a section entitled, **"CROSS REFERENCE TO RELATED APPLICATIONS"** which states the relationship between this continuation application and its corresponding parent application on which Applicants base their claim for the benefit of an earlier filing date.

Applicants have amended the claims to specifically claim preferred methods of the invention for isolating a peptide, polypeptide, or protein molecule using affinity particles in the presence of detergent to reduce particle loss during any separation step. Applicants have canceled Claims 1, 4, 9-12, 31, 33, 35, 40-43, without prejudice, which are directed to other embodiments, such as methods for separating other types of molecules. Applicants reserve the right to pursue the canceled claims in one or more continuation applications.

Applicants have amended Claims 2, 15, 32, 34, 46, 64, and 66 to clearly cover methods of the invention for isolating peptide, polypeptide, or protein molecules using affinity particles in the presence of detergent to reduce particle loss. Support for the amendments is found throughout the specification (see, e.g., p. 5, lines 19-20; p. 9, lines 15-19; p. 15, lines 22-23; and Examples 1-6, pp. 20-27). Accordingly, the amendments add no new matter.

Applicants have amended Claims 3, 13, 14, 16, 23-30, and 32 to remove dependency from canceled Claim 1. As amended, Claims 3, 13, 14, 16, 23-30, and 32 retain dependency from Claim 2. Accordingly, the amendments add no new matter.

Applicants have also amended Claims 44, 45, 47, and 54-63 to remove dependency from canceled Claim 33. As amended, Claims 44, 45, 47, and 54-63 retain dependency from Claim 34. Accordingly, the amendments add no new matter.

Claims 14 and 45 have been amended to place the claims in a proper Markush format. In particular, Claims 14 and 45 are directed to particular embodiments of the methods of Claims 2

and 34, respectively, wherein the affinity particles used in the methods are composed of any of the materials recited in a list of selected materials. In order to place Claims 14 and 45 in a proper Markush format, Applicants have deleted from the list of materials the exemplary expressions "such as polyvinyl alcohol", "including calcium, magnesium, and aluminum silicates", and "including titanium oxides, and tin oxides". The specific embodiments of the methods of Claims 14 and 45 employing each of the materials deleted from original Claims 14 and 45 are now recited separately in new Claim 67 (use of polyvinyl alcohol), Claim 68 (use of specific silicates), and Claim 69 (use of specific metal oxides). In addition to original Claims 14 and 45, support for the amendments and new claims is also found elsewhere in the specification (see, e.g., p. 5, line 29-p. 6, line 2 of the specification).

Applicants have also amended Claims 15 and 46 to clearly cover particularly preferred embodiments of the claimed invention for isolating peptides, polypeptides, or proteins using affinity particles in the presence of detergent. As noted above Applicants have added the terms "peptides" and "polypeptides" to a group of preferred possible binding partner molecules isolated by the claimed method. Applicants have also deleted recitation of the phrase "oligo-dT, nucleic acid polynucleotides complementary to a nucleic acid of interest" from the preferred list of possible affinity ligands that may coat an affinity particle and have deleted the terms "DNA, RNA, and small molecules" from the preferred list of possible binding partner molecules that may be isolated according to the claimed method. Accordingly, the amendments to Claims 15 and 46 add no new matter.

Applicants have also amended Claims 18, 19, 32, 49, 50, 62, and 63 to correct an inadvertent typographical error. Specifically, in the description of various nonionic detergents useful in the methods of the invention to reduce particle loss, the incorrectly spelled term "polyoxyehtylene" is corrected to "polyoxyethylene". Support for these amendments is found in the specification (see, e.g., p. 13, lines 15-17). Accordingly, the amendments add no new matter.

Applicants also amended Claims 27, 28, 30, 58, 59, and 61 to delete inadvertent duplications in terms in the claim language. In particular, Claims 27, 28, 58, and 59 were amended to delete one of the duplicated recitations of the article "a" before the term "cationic". Claims 30 and 61 were amended to delete one of the duplicated recitations of the phrase "is a"

before the term "zwitterionic". Accordingly, the amendments only correct errors in grammar and add no new matter.

Entry of the above amendments is respectfully requested.

Claims Pending

Upon entry of the above amendments, the claims pending in this application are Claims 2, 3, 5-8, 13-30, 32, 34, 36-39, and 44-69. The amended claims are directed to methods of isolating peptide, polypeptide, and protein molecules using insoluble affinity particles and detergent at one or more steps to reduce particle loss.

Examination and allowance of Claims 2, 3, 5-8, 13-30, 32, 34, 36-39, and 44-69 as presented herein are respectfully solicited.

Respectfully submitted,

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March 20, 2001
date

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Amended Claims with Markings Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

2. (amended) A method for isolating a peptide, polypeptide, or protein molecule from a sample in a vessel, comprising the steps of:

(a) combining the sample containing a peptide, polypeptide, or protein molecule of interest with affinity particles suitable for binding said molecule, said affinity particles being insoluble in the sample;

(b) collecting the affinity particles;

(c) separating the affinity particles from the unbound remainder of the sample;

(d) optionally, resuspending the affinity particles in a solution;

(e) optionally, eluting said molecule from the affinity particles, followed by separating the affinity particles from said eluted molecule;

wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of detergent sufficient to reduce loss of particles during any separation step, in comparison to the same method performed in the absence of detergent.

3. (amended) The method according to Claim [1 or] 2, wherein the combining step (a) is carried out in the absence of detergent, but detergent is added prior to the separation step (b).

13. (amended) The method according to Claim [1 or] 2, wherein said particles are selected from the group consisting of ferromagnetic beads, superparamagnetic beads, and combinations thereof.

14. (amended) The method according to Claim [1 or] 2, wherein said particles are composed of materials selected from the group consisting of agarose, silica, nitrocellulose, cellulose, acrylamide, latex, polystyrene, polyacrylate, polymethacrylate, polyethylene polymers, [such as polyvinyl alcohol], glass particles, silicates [including calcium, magnesium and aluminum silicates], metal oxides [including titanium oxides, and tin oxides], apatites, and combinations thereof.

15. (amended) The method according to Claim 14, wherein said particles are coated with an affinity ligand selected from the group consisting of antibodies for a particular antigen, antigens for a particular antibody, antibodies recognizing a class of molecules, streptavidin, streptavidin-tagged fusion proteins, biotin, biotin-tagged fusion proteins, glutathione, cellulose, amylose, ion exchange groups, hydrophobic interaction groups, [oligo-dT, nucleic acid polynucleotides complementary to a nucleic acid of interest,] binding molecules for cell-surface markers, phage ligands, antibodies recognizing cell or phage surface antigens, and polypeptides, nucleotides or small molecules capable of affinity interactions with a binding partner selected from the group consisting of [another] peptides, polypeptides, and proteins [, DNA, RNA, and small molecules].

16. (amended) The method according to Claim [1 or] 2, wherein said detergent, where present, is at a concentration of from about 0.0005% to 2.0% (v/v).

18. (amended) The method according to Claim 17, wherein said nonionic detergent is selected from the group consisting of polyoxyethylene (10) cetyl alcohol, polyoxyethylene (20) cetyl alcohol, polyoxyethylene (23) lauryl alcohol, polyoxyethylene (4-5) *p-t*-octyl phenol, polyoxyethylene (7-8) *p-t*-octyl phenol, polyoxyethylene (9) *p-t*-octyl phenol, polyoxyethylene (9-10) *p-t*-octyl phenol, polyoxyethylene (9-10) nonylphenol, polyoxyethylene [polyoxyehtylene] (20) sorbitol monolaurate, polyoxyethylene [polyoxyehtylene] (20) sorbitol monopalmitate, polyoxyethylene [polyoxyehtylene] (20) sorbitol monooleate, octyl-β-glucoside, APO-10, APO-12, cyclohexyl-*n*-ethyl-β-D-maltoside, cyclohexyl-*n*-hexyl-β-D-maltoside, cyclohexyl-*n*-methyl-β-maltoside, *n*-decanoylsucrose, *n*-decyl-β-D-glucopyranoside, *n*-decyl-β-maltopyranoside, *n*-decyl-β-D-thiomaltoside, *n*-dodecanoyl sucrose, and heptane-1,2,3-triol, and combinations thereof.

19. (amended) The method according to Claim 17, wherein said nonionic detergent is polyoxyethylene [polyoxyehtylene] (20) sorbitol monolaurate.

23. (amended) The method according to Claim [1 or] 2, wherein the detergent, where present, is a nonionic detergent at a concentration of at least about 0.005% (v/v).

24. (amended) The method according to Claim [1 or] 2, wherein the detergent, where present, is a nonionic detergent at a concentration not exceeding about 2% (v/v).

25. (amended) The method according to Claim [1 or] 2, wherein the detergent, where present, is an anionic detergent at a concentration of at least about 0.05% (v/v).

26. (amended) The method according to Claim [1 or] 2, wherein the detergent, where present, is an anionic detergent at a concentration not exceeding about 1% (v/v).

27. (amended) The method according to Claim [1 or] 2, wherein the detergent, where present, is a [a] cationic detergent at a concentration of at least about 0.5% (v/v).

28. (amended) The method according to Claim [1 or] 2, wherein the detergent, where present, is a [a] cationic detergent at a concentration not exceeding about 1% (v/v).

29. (amended) The method according to Claim [1 or] 2, wherein the detergent, where present, is a zwitterionic detergent at a concentration of at least about 0.01% (v/v).

30. (amended) The method according to Claim [1 or] 2, wherein the detergent, where present, is a [is a] zwitterionic detergent at a concentration not exceeding about 2% (v/v).

32. (amended) The method according to Claim [1 or] 2, wherein the molecule is a protein, polypeptide, or peptide and the detergent is polyoxyethylene polyoxyehtylene (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).

34. (amended) A method for isolating a peptide, polypeptide, or protein molecule from a sample in a vessel, comprising the steps of:

(a) providing a multiplicity of affinity particles and incubating said particles in the presence of a detergent;

(b) combining the sample containing a peptide, polypeptide, or protein molecule of interest with affinity particles suitable for binding said molecule, said affinity particles being insoluble in the sample;

(c) collecting the affinity particles;

(d) separating the affinity particles from the unbound remainder of the sample;

(e) optionally, resuspending the affinity particles in a solution;

(f) optionally, eluting said molecule from the affinity particles, followed by separating the affinity particles from said eluted molecule;

wherein any of the steps (b), (c), (d), (e) if present, and (f) if present may optionally be also performed in the presence of detergent, wherein the use of detergent is sufficient to reduce loss of particles during any separation step, in comparison to the same method performed in the absence of detergent.

44. (amended) The method according to Claim [33 or] 34, wherein said particles are selected from the group consisting of ferromagnetic beads, superparamagnetic beads, and combinations thereof.

45. (amended) The method according to Claim [33 or] 34, wherein said particles are composed of materials selected from the group consisting of agarose, silica, nitrocellulose, cellulose, acrylamide, latex, polystyrene, polyacrylate, polymethacrylate, polyethylene polymers [such as polyvinyl alcohol], glass particles, silicates [including calcium, magnesium and aluminum silicates], metal oxides [including titanium oxides, and tin oxides], apatites, combinations thereof.

46. (amended) The method according to Claim 45, wherein said particles are coated with an affinity ligand selected from the group consisting of antibodies for a particular antigen, antigens for a particular antibody, antibodies recognizing a class of molecules, streptavidin, streptavidin-tagged fusion proteins, biotin, biotin-tagged fusion proteins, glutathione, cellulose, amylose, ion exchange groups, hydrophobic interaction groups,

[oligo-dT, nucleic acid polynucleotides complementary to a nucleic acid of interest,] binding molecules for cell-surface markers, phage ligands, antibodies recognizing cell or phage surface antigens, and polypeptides capable of affinity interactions with a binding partner selected from the group consisting of [another] peptides, polypeptides, and proteins [, DNA, RNA, and small molecules].

47. (amended) The method according to Claim [33 or] 34, wherein said detergent, where present, is at a concentration of from about 0.0005% to 2.0% (v/v).

49. (amended) The method according to Claim 48, wherein said nonionic detergent is selected from the group consisting of polyoxyethylene (10) cetyl alcohol, polyoxyethylene (20) cetyl alcohol, polyoxyethylene (23) lauryl alcohol, polyoxyethylene (4-5) *p-t*-octyl phenol, polyoxyethylene (7-8) *p-t*-octyl phenol, polyoxyethylene (9) *p-t*-octyl phenol, polyoxyethylene (9-10) *p-t*-octyl phenol, polyoxyethylene (9-10) nonylphenol, polyoxyethylene [polyoxyehtylene] (20) sorbitol monolaurate, polyoxyethylene [polyoxyehtylene] (20) sorbitol monopalmitate, polyoxyethylene [polyoxyehtylene] (20) sorbitol monooleate, octyl-β-glucoside, APO-10, APO-12, cyclohexyl-*n*-ethyl-β-D-maltoside, cyclohexyl-*n*-hexyl-β-D-maltoside, cyclohexyl-*n*-methyl-β-maltoside, *n*-decanoylsucrose, *n*-decyl-β-D-glucopyranoside, *n*-decyl-β-maltopyranoside, *n*-decyl-β-D-thiomaltoside, *n*-dodecanoyl sucrose, and heptane-1,2,3-triol, and combinations thereof.

50. (amended) The method according to Claim 49, wherein said nonionic detergent is polyoxyethylene [polyoxyehtylene] (20) sorbitol monolaurate.

54. (amended) The method according to Claim [33 or] 34, wherein the detergent, where present, is a nonionic detergent at a concentration of at least about 0.005% (v/v).

55. (amended) The method according to Claim [33 or] 34, wherein the detergent, where present, is a nonionic detergent at a concentration not exceeding about 2% (v/v).

56. (amended) The method according to Claim [33 or] 34, wherein the detergent, where present, is an anionic detergent at a concentration of at least about 0.05% (v/v).

57. (amended) The method according to Claim [33 or] 34, wherein the detergent, where present, is an anionic detergent at a concentration not exceeding about 1% (v/v).

58. (amended) The method according to Claim [33 or] 34, wherein the detergent, where present, is a [a] cationic detergent at a concentration of at least about 0.5% (v/v).

59. (amended) The method according to Claim [33 or] 34, wherein the detergent, where present, is a [a] cationic detergent at a concentration not exceeding about 1% (v/v).

60. (amended) The method according to Claim [33 or] 34, wherein the detergent, where present, is a zwitterionic detergent at a concentration of at least about 0.01% (v/v).

61. (amended) The method according to Claim [33 or] 34, wherein the detergent, where present, is a [is a] zwitterionic detergent at a concentration not exceeding about 2% (v/v).

62. (amended) The method according to Claim [33 or] 34, wherein the molecule is a nucleic acid and the detergent is polyoxyethylene [polyoxyehtylene] (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).

63. (amended) The method according to Claim [33 or] 34, wherein the molecule is a protein or peptide and the detergent is polyoxyethylene [polyoxyehtylene] (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).

64. (amended) A method for isolating a peptide, polypeptide, or protein molecule from a sample in a vessel, comprising the steps of:

(a) combining the sample containing a peptide, polypeptide, or protein molecule of interest with magnetic affinity particles suitable for binding said molecule, said magnetic affinity particles being insoluble in the sample;

(b) applying a magnetic field to the vessel so as to attract and immobilize the magnetic affinity particles;

(c) separating the unimmobilized remainder of the sample from the immobilized magnetic affinity particles;

(d) optionally, resuspending the magnetic affinity particles in a solution;

(e) optionally, eluting said molecule from the affinity particles, followed by separating the affinity particles from said eluted molecule;

wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of detergent sufficient to reduce loss of particles during any separation step, in comparison to the same method performed in the absence of detergent.

66. (amended) A method for isolating a peptide, polypeptide, or protein molecule from a sample in a vessel, comprising the steps of:

(a) providing a multiplicity of magnetic affinity particles and incubating said particles in the presence of a detergent;

(b) combining the sample containing a peptide, polypeptide, or protein molecule of interest with said affinity particles suitable for binding said molecule, said affinity particles being insoluble in the sample;

(c) immobilizing the magnetic affinity particles by applying a magnet to said vessel;

(d) separating the remainder of the sample from the immobilized magnetic affinity particles;

(e) optionally, resuspending the affinity particles in a solution;

(f) optionally, eluting said molecule from the affinity particles, followed by separating the affinity particles from said eluted molecule;

wherein any of the steps (b), (c), (d), (e) if present, and (f) if present may optionally be also performed in the presence of detergent, wherein the use of detergent is sufficient to reduce loss of particles during any separation step, in comparison to the same method performed in the absence of detergent.

67. (new) The method according to Claim 14 or 45, wherein the polyethylene polymer is a polyvinyl alcohol.

68. (new) The method according to Claim 14 or 45, wherein the silicate is selected from the group consisting of calcium silicate, magnesium silicate, aluminum silicate, and combinations thereof.

69. (new) The method according to Claim 14 or 45, wherein the metal oxide is selected from the group consisting of titanium oxide, tin oxide, and combinations thereof.